



Development of an experimental method for well-controlled blast induced traumatic limb fracture in rats

Luyang Xu ^{a, b}, Xiancheng Yu ^{a, c, *}, Clement D. Favier ^b, Ibidumo Igah ^d,
Thuy-Tien Nguyen ^{a, b}, Warren Macdonald ^{a, b}, Anthony MJ. Bull ^{a, b}

^a Centre for Blast Injury Studies, Imperial College London, London, United Kingdom

^b Department of Bioengineering, Imperial College London, London, United Kingdom

^c Dyson School of Design Engineering, Imperial College London, London, United Kingdom

^d Department of Trauma and Orthopaedics, Royal Free London National Health Service Foundation Trust, London, United Kingdom

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ABSTRACT

Heterotopic ossification (HO) is a consequence of traumatic bone and tissue damage, which occurs in 65% of military casualties with blast-associated amputations. However, the mechanisms behind blast-induced HO remain unclear. Animal models are used to study blast-induced HO, but developing such models is challenging, particularly in how to use a pure blast wave (primary blast) to induce limb fracture that then requires an amputation. Several studies, including our recent study, have developed platforms to induce limb fractures in rats with blast loading or a mixture of blast and impact loading. However, these models are limited by the survivability of the animal and repeatability of the model. In this study, we developed an improved platform, aiming to improve the animal's survivability and injury repeatability as well as focusing on primary blast only. The platform exposed only one limb of the rat to a blast wave while providing proper protection to the rest of the rat's body. We obtained very consistent fracture outcome in the tibia (location and pattern) in cadaveric rats with a large range of size and weight. Importantly, the rats did not obviously move during the test, where movement is a potential cause of uncontrolled injury. We further conducted parametric studies by varying the features of the design of the platform. These factors, such as how the limb is fixed and how the cavity through which the limb is placed is sealed, significantly affect the resulting injury. This platform and test setups enable well-controlled limb fracture induced directly by pure blast wave, which is the fundamental step towards a complete in vivo animal model for blast-induced HO induced by primary blast alone, excluding secondary and tertiary blast injury. In addition, the platform design and the findings presented here, particularly regarding the proper protection of the animal, have implications for future studies investigating localized blast injuries, such as blast induced brain and lung injuries.

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1. Introduction

Blast injury is a prevalent injury in battlefields and terrorist attacks [1,2]. This is primarily due to the vast use of improvised explosive devices (IEDs) in recent military conflicts (e.g. wars in Iraq and Afghanistan), which accounted for 75% of all blast injuries. Over 70% of blast injuries involve limbs [3]. These blast-induced

extremity injuries can be produced by the exposure to a blast wave (primary blast injury), the impact of fragments/debris (secondary blast injury) and the individual's impact with surrounding objects due to body movement and structural collapse (tertiary blast injury), in isolation or in combination [4–7]. Although secondary and tertiary blast injuries are the most common sources of injury, studies have shown that the primary blast (blast wave) alone can directly cause bone fractures and is likely to be the underlying cause of limb avulsions in individuals exposed to sufficiently intense stress waves [8]. Moreover, a previous study found that the fractures resulting in major limb amputations are directly induced by the coupling of pure shock waves [9].

* Corresponding author. Centre for Blast Injury Studies, Imperial College London, London, United Kingdom.

E-mail address: x.yu16@imperial.ac.uk (X. Yu).

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Heterotopic ossification (HO) is a frequent complication in post-traumatic healing after musculoskeletal trauma such as fractures, blast injuries, and arthroplasties, often associated with severe pain and poor prognosis [10,11]. The traumatic damage of skeletal and muscle cells, as well as cellular and tissue hypoxia can trigger a local inflammatory response and enhance several signal pathways, including hypoxia-inducible factors signaling and bone morphogenetic protein signaling [12]. These signals inappropriately activate an osteogenic or osteochondrogenic program in the muscle and soft tissues [5,13]. However, a comprehensive knowledge of the etiology of HO is still elusive. The prevalence of HO distinctly differs between civilian patients and combat casualties [14]. The average occurrence of HO in combat casualties who underwent at least one orthopedic procedure is 65%, and is higher for patients suffering lower extremity external fixation or amputation procedures [15], the vast majority of whom had blast-induced injuries. This figure is much higher than that of non-blast patients with extensive surgery trauma, whose HO occurrence rate is only 30% [16]. Extremity injuries due to secondary and tertiary blast mechanisms can be considered as similar to civilian impact-induced extremity injuries, such as due to road traffic collisions (RTC), the key distinction between civil and battlefield injuries is the presence of the blast wave. Therefore, there is a necessity to develop methods to study the blast wave effect on HO formation in blast-injured amputees.

One approach to study the effect of blast on human HO is to use animal models. To replicate the blast-induced HO in combat individuals, the animal models need to include three essential risk factors: using blast wave as loading, producing limb fracture and performing amputation [15]. The first and most challenging step is how to induce limb fracture using a pure blast wave. There have been several studies developing such animal models, using blast wave generated either from explosive detonation or a shock tube [17–19]. These studies have used two methods to model the blast-induced limb fracture: (1) the animal was exposed to a blast wave without limb fracture. Next, the animal's leg was fixed at two ends and fractured by dropping a weight onto the center of the leg [17] or (2) the animal was fixed above a tank with its leg exposed. The tank was filled with sand or water and explosive was placed at the bottom of the tank covered by sand or water. Upon detonation, the sand or water was accelerated upwards to impact the animal's leg, causing limb fracture [18,19]. These studies have provided invaluable information about blast-induced HO. However, they are limited by the fact that the limb fracture was not caused by pure blast wave exposure, but by impact loading similar to the civilian limb fracture cases. Therefore, it is unclear whether the HO formation is a result of the blast wave effect (primary blast injury) or joint effect of blast wave and impact (primary and tertiary blast injury).

To study this further, a rodent blast injury model, aiming to induce limb fracture only by a blast wave was developed previously by our group [20]. The animal was mounted within a cradle with its leg exposed to a blast wave. However, this model has limitations: (1) the repeatability of the animal's injury is poor. Changes in rat size, fixation, and positioning can significantly affect the resultant injury (e.g., induction of fracture, location and type of limb fracture); (2) a large part of the animal's body was exposed to the blast wave, inducing multiple unplanned injuries; and (3) the mechanism of limb fracture used in this model may not accurately represent primary blast injury, where fractures are directly induced by the coupling of pure blast wave. The animal was placed within a seat with one of its legs hanging and exposed to blast wave. The high-speed videos showed that the animal was accelerated upward and its lower extremities impacted with the cradle, suggesting that the limb fracture was induced by the impact rather than the blast. Therefore, this platform may not be able to induce limb fracture by

pure blast wave exposure.

The aim of this study is to address these issues by redeveloping our test platform and optimizing the test setup, in order to replicate the mechanism of primary blast induced extremity injuries (fractures) in the open-field battlefield explosions. We resolved the problems in the previous rat injury model and achieved (1) consistent limb fractures with excellent repeatability, and (2) enhanced protection to the rat body avoiding additional injuries. This model will be further translated to living animals to develop a complete blast-induced HO animal model, which will include amputation surgery (through the fracture zone) to investigate the blast wave effect on HO formation.

2. Method

2.1. Animals and specimens

The experiments were designed in compliance with the UK Animal (Scientific Procedures) Act 1986. All the procedures were approved by the UK Government Home Office and were carried out in compliance with animal welfare regulations. In line with the 3R principles (replacement, reduction, and refinement) in animal research, we used cadaveric rats sacrificed prior to the tests. A total of 40 male Sprague-Dawley rat cadavers (weighing 230–523 g) were used in this study (Fig. 1(d)). Of these, 32 rats were tested using the improved platform with ideal test setup, which was to test the repeatability and performance of the improved platform. The other 8 rats were used to investigate how different design factors of the test setup can affect the limb fracture results.

2.2. The shock tube test rig

We used the shock tube test rig (Fig. 1(a)) at the Centre for Blast Injury Studies (CBIS) at Imperial College London [21] to conduct the blast tests. The shock tube has an inner diameter of 59 mm and a length of 3.8 m. It was designed as a two-chamber air-driven system using Mylar films with different thicknesses, allowing the generation of blast waves of different intensities. The driver section of the shock tube was pressurized with compressed air to a desired pressure. Then, the mylar film was ruptured at the burst pressure, which released the compressed air producing a blast wave propagating along the driven section and impact on the animal placed at the outlet of the shock tube [20]. We used the burst pressure to distinguish different blast waves. It is possible to control the positive phase duration by changing the volume of the driver section, but we used the full volume of the driver section in this study, which maximizes the positive phase duration. Peak pressure and positive phase duration used in our study are representative of those seen in the battlefield [22]. However, it should be noted that the blast waves used in this animal model are not scaled from those in the battlefield using the animal-to-human scaling law. Instead, we select the level of blast wave loading to match the severity of lower extremities injuries between animal and human. As HO is usually seen in combat casualties who underwent lower extremity amputation procedures following limb fracture (Forsberg et al., 2009), we selected blast waves that can induce limb fracture in rats. The theory and calibration details of the shock tube can be found in our previous studies [22–25].

Two dynamic pressure sensors (Dytran® model 2300V3, range: 34.5 bar) were mounted along the shock tube's driven section, which were used to trigger a datalogger (sensor 1) and measure the incident blast wave applied to the animal (sensor 2). These two pressure signals were sampled by an oscilloscope at 50 MHz. The incident blast wave pressure data (Fig. 1(c)) presented in this study were filtered with a 70 kHz Butterworth low-pass filter to eliminate

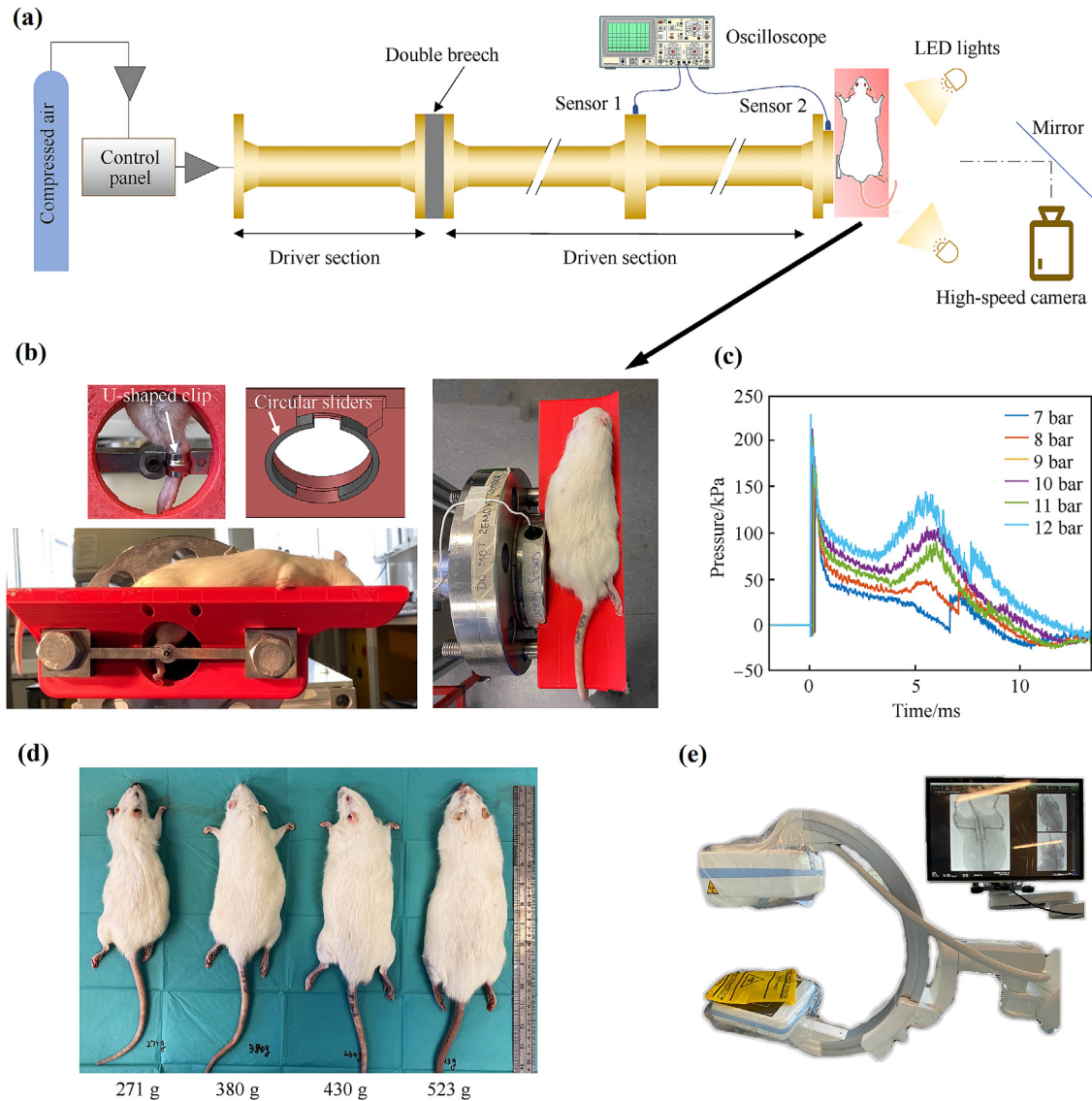


Fig. 1. (a) The shock tube test rig and test setup; (b) The animal platform and the animal fixation. The circular sliders are placed within the circumference of the chamber, which did not block the blast wave transmission; (c) The blast wave loading profiles; (d) Rats of different masses (271–523 g); (e) Inspection of the rats by X-ray.

random noise in the signal, as recommended in Ref. [22]. The peak overpressure values produced from the same burst pressure are very similar with small coefficient of variation ($<1\%$). The incident blast wave has a second peak at around 6 ms, in addition to the shock front (first peak), which is due to the double chamber design of the shock tube. Such waveform is slightly different to the ideal Friedlander waveform, which has a shock front (instant pressure rise) followed by an exponential pressure decay. In real world blast cases, the waveforms of blast waves can be affected by the surrounding environment (e.g., nearby structures), thus they are often different to the ideal Friedlander waveform.

A high-speed video camera (Phantom VEO 710L triggered by sensor 2) was used to film the tests. The camera's frame rate was set at 72,000 frames per second, capturing images with 320×240 pixels. We used high-power Light Emitting Diodes (LEDs) to provide lighting (Fig. 1(a)).

2.3. The improved animal platform

Fig. 1(b) shows the improved animal platform. This platform

enables the rat's hindlimbs alone to be exposed to the blast wave, while providing proper protection to the rest of the body. The 3D printed platform was designed with a flange and can be fitted to the outlet of the shock tube, aligning the platform's central hole with the shock tube. The rat was laid on the upper deck of the platform in a prone position. The rat's left leg was suspended through a hole, with its lateral side facing the blast wave. The size of the hole is $5 \text{ cm} \times 1 \text{ cm}$, which can accommodate rats with body mass over 500 g.

A key feature of this design is the two circular sliders that seal the hole. These sliders are placed within the circumference of the chamber. After fixing the rat leg, we moved the two sliders to seal the hole to minimize the hollow space around the leg. This design was made based on previous experience. The rat leg and structures obstructing the blast wave can alter the blast direction and one part of the blast wave may propagate upwards and damage the abdomen of the rat. Therefore, sealing the hole with the sliders can resist the altered blast wave.

The rat's proximal tibia was constrained within the hole while the ankle was fixed with a U-shaped clip onto a steel beam

(Fig. 1(b)). With these proximal and distal fixations, the blast wave applied to the leg induces limb fracture in the middle of the diaphysis of the tibia, which follows the three-point bending fracture mechanics. It should be noted that fixing the leg at the ankle maximized the area of the leg exposed to the blast wave and also increased the distance between the two fixations. According to the three-point bending theory, this can generate a higher bending moment, thus increasing the possibility of fracture.

With this setup, we tested 32 rats with different body masses ranging from 230 g to 523 g (Fig. 1(d)). We also varied the blast wave loadings by using different burst pressures (7, 8, 9, 10, 11 and 12 bar) (Fig. 1(c)).

2.4. Parametric studies for investigating the effect of platform designs

In the above test setup, we sealed the hole of the platform with the sliders and fixed the rat leg at the ankle. To investigate the effect of these two design features separately, we further conducted two groups of tests. In one group, we kept all the test settings the same, except that the hole was open (not sealed by the sliders) (Fig. 2(a)). In the other group, we kept all the test settings the same, except that we fixed the rat leg at the distal tibia (not the ankle), which reduced leg's area exposed to the blast wave (Fig. 2(b)).

2.5. Injury identification

We used the Fluoroscanner InSight FD Mini C-Arm system (Hologic, USA) (imaging parameters: 49 kV, 0.056 mA) to image the rat limbs after the tests (Fig. 1(e)). The bone fracture was determined by the X-ray images. We also inspected the high-speed video footage to evaluate the movement of the rat's body, a surrogate measure of the blast wave impacting elsewhere on the rat's body. This is particularly important for the abdomen, where the abdominal organ injury and internal bleeding resulting from the propagation of the blast wave is a major cause of death in animal blast injury studies [26]. In this study, we consider the additional fractures (limb fractures outside of the target area) and the potential abdominal injury, identified through X-ray and high-speed video, as additional injuries in our measurements.

3. Results

3.1. Limb fractures and body movement

With the improved animal platform, we tested rats of varying body masses (230–523 g) at different burst pressures (7–12 bar). Fig. 3(b) shows the limb fracture results of these tests. All the tests at 8–12 bar burst pressure produced bone fracture in the diaphysis

of the tibia for all rats (Figs. 3(a)–3(c)), with the tissue damage confined to the level of fracture. Burst pressure of 7 bar produced limb fracture in the smallest rats (<230 g), but it did not induce limb fracture on larger rats (>250 g) (Fig. 3(b)). This suggests that both rat size and blast wave intensity are likely to affect limb fracture.

Fig. 3(a) compares the limb fracture of two rats with different size under the same burst pressure (12 bar). Both rats have only one fracture at the desired location, approximately the middle diaphysis of the tibia. Fig. 3(c) compares the limb fracture of rats with similar size (360 ± 10 g) under different burst pressures. All burst pressures over 7 bar produced limb fracture at the middle of the tibia.

Next, we inspected the high-speed video footage of two blast tests using 8 bar and 12 bar burst pressures (Fig. 3(d)). In both cases, the rat's body has no obvious displacement. There is no obvious difference in limb kinematics in the two cases. The limb reached its maximum displacement at around 2.3–2.4 ms, followed by the several rounds of back-and-forth movement. The blast wave exposure lasted around 15 ms and the limbs stopped moving at around 90 ms. These two high-speed videos are included in the supplementary material (Appendix A: Video A1 and Video A2).

3.2. The effect of hole's sealing condition on limb fracture

Next, we investigated the effect of hole's sealing condition on the limb fracture, as well as the protection to the rest rat's body. We conducted blast tests without using the circular sliders to seal the hole (Fig. 4(a)), namely the hole was open. Four rats with different body masses (357–431 g) were exposed to blast wave produced by 12 bar burst pressure and the leg was fixed at ankle. Radiographic results confirmed that no limb fracture was induced in any case (Figs. 4(b)–4(c)). However, the tests using the same blast wave and similar rat size but with the hole sealed all induced limb fracture (Fig. 3(a)). This suggests that the open hole reduced the loading applied to the limb, which could be due to that a large amount of blast wave vented from the hole. The snapshots of the high-speed video footage (Fig. 4(d)) further confirm this: a large amount of the blast wave propagates from the hole and impacts the rat's body. The body reached its maximum displacement at around 90 ms and stopped moving at around 200 ms (Appendix A: Video A3). This is different to the hole-sealed condition, where the rat body was almost static during the blast exposure (Fig. 3(d)).

3.3. The effect of fixation location on injury result

Finally, we studied the effect of the fixation location on limb fracture. Fig. 5(a) shows that the rat leg was fixed at the distal tibia, shorter than those fixed at the ankle (Fig. 2(b)). Four rats weighing

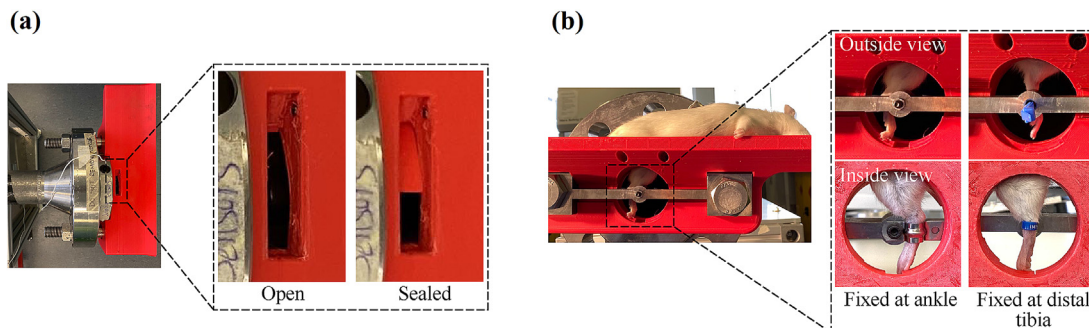


Fig. 2. Comparison of (a) the hole status: open vs sealed and (b) the position of leg fixation.

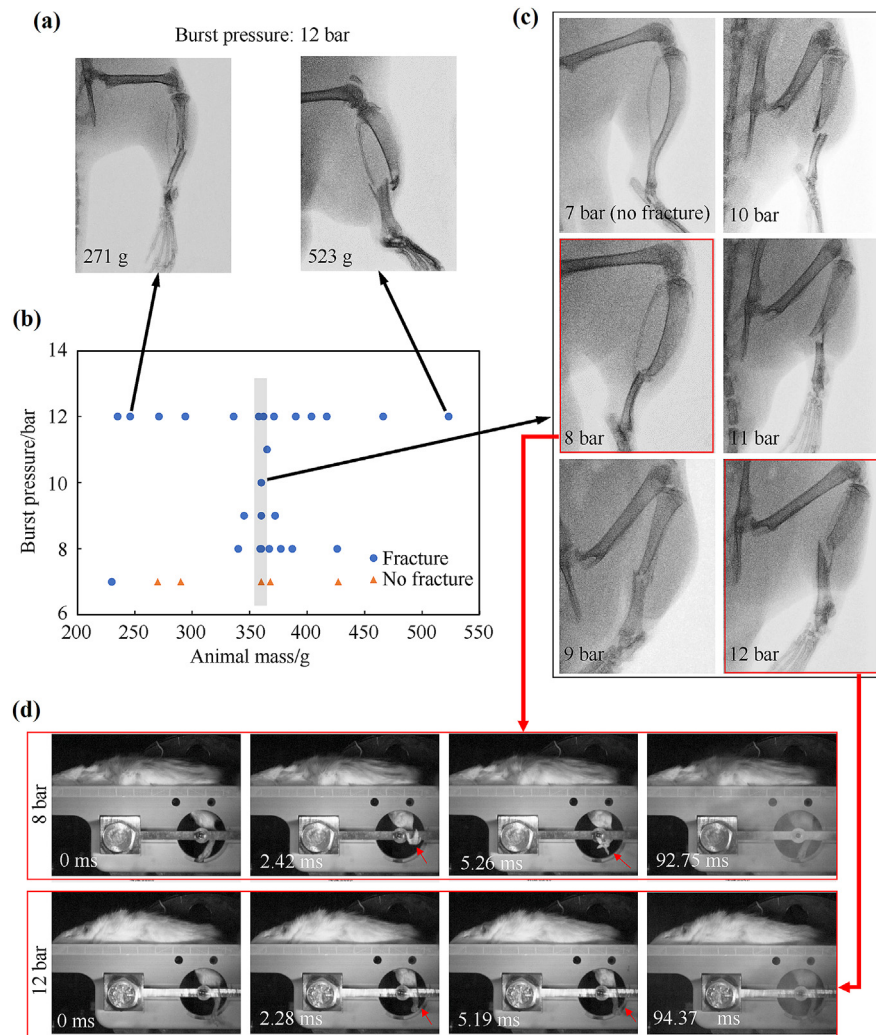


Fig. 3. Blast test results using the improved animal platform: (a) Limb fracture comparison of rats with different body masses (271 g and 523 g) under the same burst pressure (12 bar); (b) Limb fracture results of the 32 rats; (c) Limb fracture comparison of rats with similar mass (360 ± 10 g) under different burst pressure; (d) High speed video footage stills of rats under 8 bar and 12 bar burst pressures.

between 345 g and 400 g were tested under blast wave generated from 12 bar burst pressure. No fracture was observed in any test (Figs. 5(b)–5(c)). Compared to the cases using ankle fixation, two factors may contribute to the no limb fracture results here. First, with ankle fixation, the ankle is clamped to the side of the crossbar, maximizing the leg's area exposed to blast wave and therefore maximizing the force acting on the leg. Secondly, the longer distance between the two fixation locations further increases the bending moment acting on the limb. Therefore, fixing the leg at the distal tibia reduces both the force and the bending moment, resulting in no limb fracture. The high-speed video footage (Fig. 5(d)) showed that the rat's body was static during the test, further confirming that sealing the hole prevents the blast wave from impacting the rat's body.

3.4. Comparison with our previous platform

Fig. 6 compares the improved platform with the platform in our previous study [20]. In our previous platform, the rat was mounted within a cradle with its left leg exposed to the blast wave (Fig. 6(a)). The left leg was only supported by an anvil at the tibia, which was supposed to induce the fracture. The other leg was protected by a

shield. The rat was loosely seated within the cradle. Such a design provided a very weak constraint to the rat, and thus allowed large movement. Furthermore, the blast wave was blocked by the shield, which was originally designed to protect the right leg. This changed the one-dimensional wave into a spherical propagating wave, impacting the rat's abdomen and accelerating the rat body (Fig. 6(a)). Due to the loose constraint, the rat's limb and hip would move up and impact onto the steel cradle, leading to additional fractures at several locations (X-ray impact in Fig. 6(a)). These fractures were also inconsistent, as number and location varied across tests. Moreover, as the cradle seat was not sealed, the blast wave would easily reach the abdomen from the hole (Fig. 6(a)). This would cause severe injuries to the abdomen, leading to poor survivability. One evidence for such injury is the intraabdominal gas seen in the X-ray image (Fig. 6(a)).

In contrast, the new platform developed here only exposes the left leg to the blast wave with proximal tibia and ankle tightly fixed (Fig. 6(b)). This leads to a consistent fracture (location and pattern) in the middle of the diaphysis of the tibia induced only by the blast wave, harnessing a three-point bending mechanism. In addition, we designed proper sealing around the leg to protect the rat's abdomen against the upward-propagating blast wave. This can be

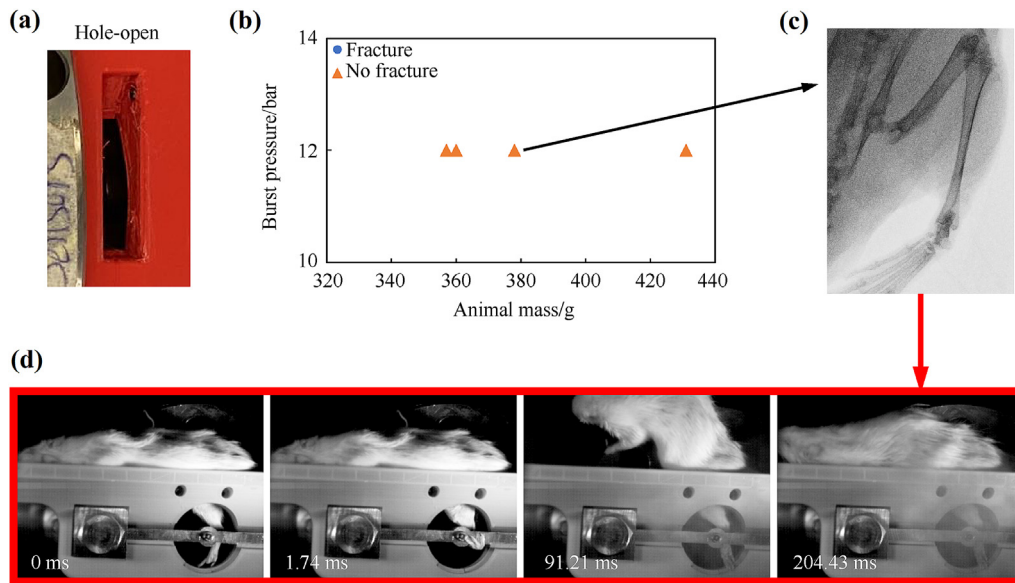


Fig. 4. Blast tests result using the hole-open condition: (a) The hole in open condition; (b) Limb fracture results; (c) X-ray images of one test showing no limb fracture; (d) High speed video footage stills of one test showing rat's body being moved by the blast wave.

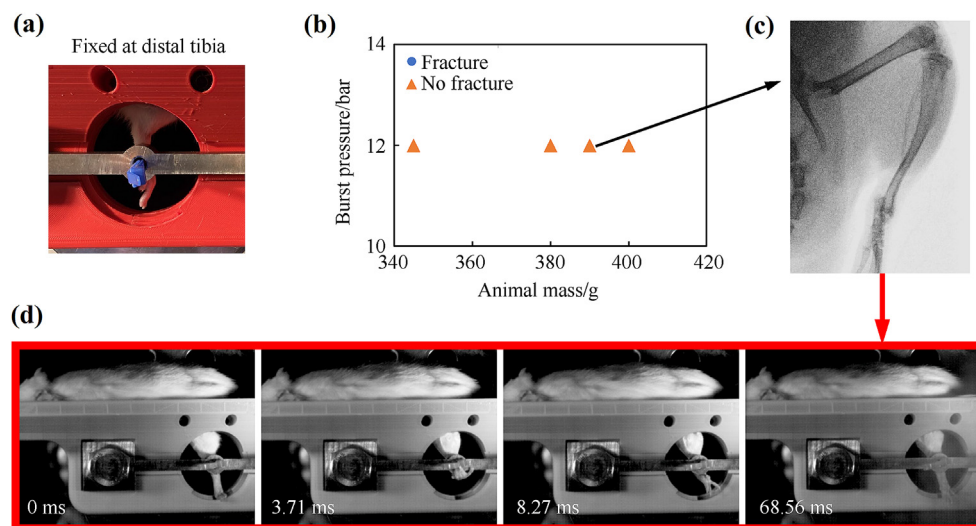


Fig. 5. Blast tests result using distal-tibia fixation: (a) The fixation at the distal tibia; (b) Limb fracture results; (c) X-ray images of one test showing no limb fracture; (d) High speed video footage stills of one test showing no bodily movement.

confirmed from the high-speed video, which shows that the rat's body is static during the test (Fig. 6(b)).

Table 1 shows a detailed comparison of test results under 12 bar burst pressure using the two platforms. The improved platform achieved a 100% success rate for the intended left tibia fracture without additional injuries. With the previous platform, all tests produced additional fractures, such as above knee fracture and right limb fracture. In addition, the improved platform can accommodate a much larger range of body mass (230–523 g) while the previous platform only accommodated medium-sized rats (307–430 g) [20].

4. Discussion

In this study, we redeveloped our blast injury model using a 3D printed platform to induce limb fracture following the 3-point

bending mechanism. This platform achieved highly repeatable and survivable blast induced musculoskeletal injury. We obtained very consistent limb fracture results in rats with a large body mass range (230–523 g) and under different burst pressures (8–12 bar). We also showed that the hole-sealing condition and limb fixation position significantly affect the limb fracture results. This platform provides a controlled pre-surgery condition for our future in-vivo studies, which focus on amputation surgery through the fracture zone and investigating the HO pathologic process near the amputation site. Furthermore, it allows us to control and quantify the intensity of the blast wave applied on the rat.

For the first time, our improved platform replicates the extremities fractures from a pure blast wave (primary blast injury). Primary blast loading is a potential reason for the high HO rate in post-traumatic healing after severe fractures. Unlike blunt impact-induced limb fracture, the blast wave interacts with human tissue

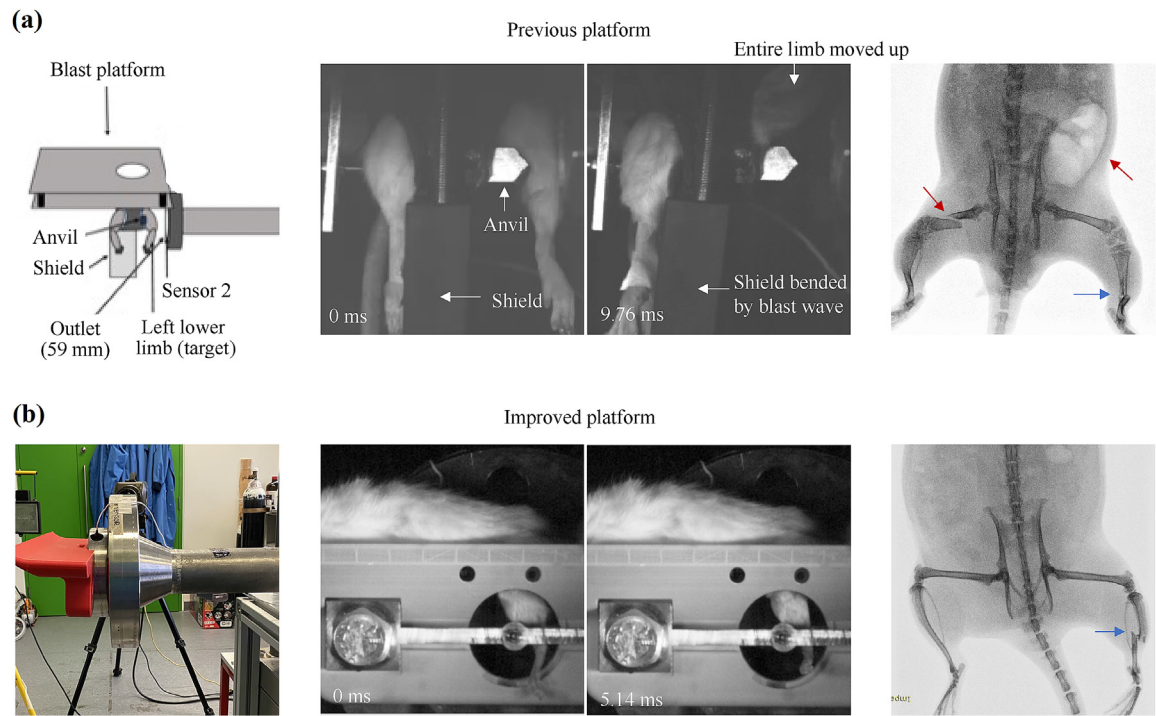


Fig. 6. Comparison between the previous platform and the improved platform (365 ± 10 g rats under 12 bar burst pressure): (a) The setup of the previous platform; high speed video footage stills showing rat's body was moved up during the test; X-ray showing additional fractures in the limbs (red arrow) other than the aimed fracture (blue arrow), and free air in the abdomen (red arrow); (b) The setup of the improved platform; high speed video footage stills showing the rat body is static during the test; X-ray showing the aimed fracture (blue arrow) in the middle of the diaphysis of the tibia.

Table 1
Comparison of test results (both under 12 bar burst pressure) between the improved platform and the platform in our previous study [20].

Platform	Rat mass range/g	Body movement during blast exposure	Fracture results (n/test number)		
			Intended left tibia fracture	Above knee fracture (left)	Right limb fracture
Improved platform	230–523	No obvious movement	13/13	None	None
Previous platform [20]	307–430	Large body movement	8/8	8/8	8/8

in a very different way. Blast wave is a supersonic pressure wave with an instant rise [23,25]. It applies dynamic pressure to the limb's surface near the explosion. These surface pressures are then coupled into the limb, creating stress waves within it. The pressure generates an impulse acting on the relative movement of limb's constituent parts (bone and tissue) [9]. It can cause shearing, spalling and compression on bone and soft tissues [27–29]. Previous studies have highlighted the distinct nature of primary blast injury compared to secondary (fragment impact) and tertiary blast injury (body impact), with both systemic and local response being activated from blast wave. For example, in blast-induced brain and lung injury, primary blast injury has been found to instigate the most prominent systemic inflammation, neuroinflammation and systemic hypoxia, compared to the response observed in other blast injuries [30–33]. Such systemic response, accompanied by local inflammation and tissue hypoxia, has also been observed in recent studies investigating HO formation in extremity injury [11,13,34].

In the primary blast injury, the molecular mechanism in blast-injured tissues (local soft tissue and fractured bone) may involve the rapid hypoxia of skeletal and muscle tissue cells, leading to an immediate inflammatory response and overproduction of cytokines (such as hypoxia-inducible factors) [34]. These cytokines play a crucial role in stimulating the differentiation of local mesenchymal cells into chondrocytes and osteoblasts, thereby activating

the osteogenesis process and facilitating ectopic bone formation (HO) [35,36]. For the persistent systemic inflammatory response throughout the whole body, it is likely initiated by tissue hypoperfusion and can progress to poor tissue oxygen delivery, leading to dysfunction of remote organs in casualties [37]. However, the mechanism has not yet been fully understood. This system response might differ from other blast injuries; it is known that secondary injuries (deep open wounds) and tertiary injuries (extensive blunt trauma) leading to hemorrhage and extensive ischemia can also contribute to the systemic response [37]. In contrast, primary blast injuries typically have relatively small soft-tissue damage, making it challenging to understand how they can trigger a systemic response. Therefore, investigating the effect of primary blast injury on HO formation, while minimizing the confounding influence of secondary and tertiary injuries, is crucial to gaining insights into the initiation of HO. However, previous studies that investigated blast HO have failed to induce bone fracture with the blast wave only. Instead, bones are fractured by non-blast (e.g., blunt impact) or mixed mechanisms [17,20]. Therefore, the isolated effect of the blast wave on HO formation remains unclear. Our improved platform can be used in future studies aimed at investigating the effects of pure blast wave exposure (primary blast injury) on HO formation, without the confounding influence of secondary and tertiary injuries.

Our platform allows limb fracture in rats to be induced with a mechanism similar to primary blast induced extremity injuries seen in the open-field battlefield explosions. The blast wave can induce limb fracture through different mechanisms [38]. For example, in our previous platform, we used an anvil to support the tibia with the lower leg free to move. During blast exposure, the leg struck upwards on the steel cradle causing fractures. Composite blast injury (primary and tertiary blast injury) is created when the animal is suspended and or protected inappropriately [32]. However, it differs from the open-field blast situation, where fractures can occur due to the direct coupling of blast waves (primary blast injury). In open-field casualties, the injuries (fractures) tend to occur preferentially through bone shafts rather than joint dislocations (typically seen in avulsion injuries) [9]. Furthermore, primary blast injuries alone have relatively little open wound of soft-tissue damage and are primarily confined to the level of fracture (amputation) area. This is in contrast to secondary blast injuries, where fragments are driven deep into the soft tissues, and tertiary blast injuries, which result from crush injuries and extensive blunt trauma [39]. In the battlefield, soldiers exposed to open-field blast are usually in the standing position, where the leg is constrained by the body weight. It can be assumed that the foot and the hip are fixed during the short-time of blast exposure (<30 ms). In such loading condition, the limb fracture is caused by three-point bending. Here, our improved platform follows this mechanism. We fixed the left limb proximally at the proximal tibia and distally at the ankle in the platform and only exposed the left limb to the blast wave, to achieve the primary blast injury in the left tibia without extra blunt injury during the exposure. While the fracture mechanism seen in both secondary and tertiary injuries may also follow a 3-point bending mechanism, it is important to note that these injuries are induced by dynamic localized loading from indirect means such as shrapnel fragments or body against an obstruction, rather than the blast wave itself [38]. In our model, the consistent tibia fracture results are specifically induced by the primary blast wave, which applies dynamic uniform loading and follows a 3-point bending mechanism.

A unique advantage of our improved platform is the good protection of the rat's body during the blast, which was confirmed by the two observations: (1) Minimal body movement shown in high-speed video footage (suggesting minimal blast damage on the rest of the rat's body); (2) X-ray images did not show intraabdominal gas (suggesting minimal organ damage) and unplanned bone fracture outside the targeted location. The body movement is shown as a leading cause of secondary body injury (e.g. unplanned bone fractures) in our previous study [20]. For organ damage, a previous study showed that blast wave can induce acute organ (especially lung, brain, and liver) injury, leading to subsequent hemorrhagic shock and multi-organ failure [26]. This may explain the high mortality rate reported in previous blast-induced HO studies [18]. Therefore, it is very important to avoid additional injuries to organs and areas outside the target. Our improved platform minimized both body movement and secondary blast injuries. Therefore, we suggest that our improved platform could reduce the mortality of rats, but the real mortality rate needs to be confirmed in our following studies using living animals. Furthermore, the concept and methods of body protection of our platform can also inform other blast injuries experiments, such as blast brain injury and blast lung injury, by providing a guideline for proper protection of the area outside the target. For instance, current studies on blast brain injury either do not protect or use shields to protect the animal's body below the head, which may lead to additional injuries to other regions. Our platform could provide insights on the design of such shields, as well as some important design features, such as how the shield's opening around the body can be sealed to impede

the transmission of the blast wave.

Our parametric studies suggested two factors that can affect the fracture results. As the limb fracture was induced by the force applied on the leg, we demonstrated that the applied force can be affected by two factors. One is the hole on the platform, where the blast wave can dissipate; therefore the load applied on the leg is reduced. The other factor is the distance between the two fixations. We showed that shifting the fixation from the ankle to distal tibia results in no limb fracture. This was because that the area exposed to blast was reduced, lowering the force and bending moment at the middle of the tibia, resulting in a lower likelihood of fracture. These principles can also be generalized to inform future studies that develop larger animal models for blast-induced bone fractures.

Our model allows for a more controlled examination of realistic primary blast injury with high repeatability. This resolves the concerns of translational studies in blast injury research, not only between cadaver and living animal studies, but also between animal data and real-life blast events [40]. This rat model of blast-induced limb fracture paves the way for future *in vivo* studies that enable an amputation through the limb fracture area to be performed. By excluding the confounding effects of secondary and tertiary injuries, this model represents the human situation in primary blast caused extremity injuries during an open-field blast wave exposure. In our further living animal study, if the isolated pure blast wave-induced fracture does not lead to HO formation, this may indicate that the blast wave may no longer be necessary for inducing injury in combat related HO, or that other injurious loading mechanisms need to co-exist. Our model provides an opportunity to investigate various other factors such as initial treatment options (negative pressure wound therapy), and surgical options such as debridement approaches on HO formation in pure blast wave condition.

There are some limitations in this study. First, we varied the blast wave intensity by using different burst pressures, which mainly changed the pressure magnitude of the blast wave, but not the positive phase duration. We did not study the effect of positive phase duration on limb fracture as the current study focuses on developing an experimental method, which uses pure blast wave to induce limb fracture. Previous studies have shown that both the pressure magnitude and positive phase duration can affect the injury severity [20,24], both of which may affect the HO initiation. Our future *in-vivo* study should investigate the effect of positive phase duration on HO formation by changing the volume of the shock tube's driver chamber. Secondly, although we focused on protection of the rat's body and assessed secondary injury by inspecting whether there is abdominal free air cavity or unplanned body movement during the tests, there may still be minor injuries in the rat's body by limited blast wave intrusion through the hole edges. We should further investigate the existence of these potential injuries through long-term measurements and animal dissection in future tests using living rats. Thirdly, as limb fracture are induced locally, the rats' tibial properties (e.g. muscle and bone strength) should be the most ideal measures to represent the effect of animal size on limb fracture. However, we are not able to determine the tibial properties due to the lack of suitable facilities. Therefore, we used rat mass as a surrogate measure to represent the effect of animal size. This decision was made based on the assumption that tibial properties are in a linear relationship with the animal mass as the rats used in this study are from the same species, same sex (male) and raised under the same condition.

In summary, we introduced an improved platform, which provided proper protection to the rat's body and achieved well controlled rat's limb fracture directly caused by pure blast wave exposure. Our designs can provide a guideline for future blast injury studies, including those for other organs such as lung or brain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dt.2023.08.003>.

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